

N73-16065-

Spectral Characteristics of Normal and  
Nutrient-Deficient Maize Leaves<sup>1</sup>

by

A. H. Al-Abbas

R. Barr

J. D. Hall

F. L. Crane

and

M. F. Baumgardner<sup>2</sup>

CASE FILE  
COPY

<sup>1</sup> Journal paper No. 4839, Purdue University Agricultural Experiment Station, Contributed by Laboratory for Applications of Remote Sensing, Dept. of Agronomy, and Dept. of Biological Sciences, West Lafayette, Indiana, 47907. Supported by: U.S.D.A. Contract #12-14-100-10292 (20), NASA Grant NGR 15-005-112, N.S.F. Grant GB5701, National Institute of General Medical Science Career Grant K6-21839, and Training Grant GM01392. Received August 10, 1972.

<sup>2</sup> Research Agronomist, Research Associate, Former Graduate Assistant, Professor in Biology, and Associate Professor of Agriculture.

## ABSTRACT

Reflectance, transmittance and absorbance spectra of normal and six types of mineral-deficient (N,P,K,S,Mg and Ca) maize (*Zea mays* L.) leaves were analyzed at 30 selected wavelengths along the electromagnetic spectrum from 500 to 2600 nm. Chlorophyll content and percent leaf moisture were also determined. Leaf thermograms were obtained for normal, N- and S-deficient leaves.

The results of the analysis of variance showed significant differences in reflectance, transmittance and absorbance in the visible wavelengths among leaf numbers 3, 4, and 5, among the seven nutrient treatments, and among the interactions of leaves and treatments. In the reflective infrared wavelengths only treatments produced significant differences.

The chlorophyll content of leaves was reduced in all deficiencies in comparison to controls. Percent moisture was increased in S-, Mg- and N-deficiencies. Positive correlation ( $r = 0.707$ ) between moisture content and percent absorption at both 1450 and 1930 nm were obtained. Polynomial regression analysis of leaf thickness and leaf moisture content showed that these two variables were significantly and directly related ( $r = 0.894$ ). Leaves from the P- and Ca-deficient plants absorbed less energy in the near infrared than the normal plants; S-, Mg-, K-, and N- deficient leaves absorbed more than the normal. Leaf thermograms were prepared on normal, S- and N-deficient leaves. Both S- and N-deficiencies produced higher temperatures than normal maize leaves.

Additional Key Words: Reflectance, Absorbance, Transmittance, Leaves Chlorophyll Content, Leaves Moisture Content, Leaves Thickness, Leaves Temperature, Leaves Thermogram.

## INTRODUCTION

The spectral characteristics of plant reflectance and transmittance are functions of leaf geometry, morphology, physiology and biochemistry. They are also influenced by soil and climatic conditions (6).

Nutrient status is one of many factors which affect plant growth. Excess or deficiency of an essential element may cause visible growth abnormalities in pigmentation, size and shape of leaves and the appearance of various other symptoms. It has been reported that in plant canopies such abnormalities can be detected spectrally and remotely (5).

Previous studies in the general area of leaf spectral reflectance and transmittance include the effect of physiological age (9), moisture content (13, 22), osmotic stress and salinity (11), pigment composition (3), the relation of cell structure upon individual leaf spectra (8, 10, 20), and plant nutrient stress (4, 14, 19, 23).

The objective of this research was to study the spectral characteristics of normal and nutrient-deficient (-N, -P, -K, -S, -Ca, -Mg) maize (*Zea mays* L.) leaves. The results of this study should provide basic knowledge for the interpretation of spectral measurements from air- and space-borne sensors.

## MATERIALS AND METHODS

Maize plants were grown in the greenhouse in seven different nutrient culture solutions as follows: normal, -N, -P, -K, -Ca, -Mg, and -S using Hoagland and Arnon's nutrient solutions (13). EDTA-chelated Fe was substituted for  $\text{Fe}_2(\text{SO}_4)_3$  as the source of iron. Inert volcanic glass called "Krum" was used for root support (obtained from the Silbrico Corp., 6300 River Road, Hodgkins, Illinois 60825).<sup>3</sup> A more detailed description of growing maize plants for this experiment is reported by Barr *et al.* (4).

---

<sup>3</sup> Use of a company or product name in this paper does not imply its approval or recommendation to the exclusion of others that may also be suitable

Eight weeks after emergence leaf sections 10-12 cm long from the broadest part of every leaf from five plants of normal and the nutrient-deficient treatments were prepared for analysis. Total reflectance and transmittance spectra were obtained over the 500-2600 nm wavelength interval with a Beckman DK-2A spectrophotometer using barium sulfate as standard.

Fresh weight and area of each leaf section were determined immediately after completing the DK-2 spectral measurements. From these data, leaf thickness (defined in this paper as weight/unit area) were calculated. The dry weights of these leaves were also determined after drying leaf sections in an oven at 100°C for 48 hours. The difference between wet and dry weights was used to calculate percent moisture.

The average chlorophyll content was determined from a pool of 5 to 6 fresh leaves of the same chlorophyll age after extraction of 5 g of leaf material with 80% acetone (1). Chlorophyll values are reported here on a fresh weight basis (15).

The DK-2 graphs for both reflectance and transmittance spectra were reduced in a manner similar to that reported by Johannsen (13). All the data, after correction to give absolute values, were punched on cards and transferred to magnetic tape to facilitate handling and storage. The thirty wavelengths selected were: 500, 530, 600, 640, 700, 740, 830, 900, 940, 1000, 1100, 1160, 1200, 1300, 1400, 1430, 1450, 1500, 1550, 1600, 1700, 1770, 1800, 1930, 2000, 2100, 2180, 2300, 2400, and 2600 nm.

Absorbance was also calculated from the absolute values as:

$$\text{Absorbance} = 100 - (\% \text{ reflectance} + \% \text{ transmittance})$$

Reflectance, transmittance and calculated absorbance data for the seven nutrient treatments of leaves 3, 4, and 5 were analyzed using analysis of variance, and the Scheffe test (18) for multiple comparisons. Fourteen selected wavelengths (530, 640, 830, 940, 1100, 1200, 1400, 1430, 1450, 1550, 1700, 1930, 2000, and 2180 nm) out of the thirty recorded were used in this analysis.

A thermoscope<sup>4</sup> was used to obtain thermograms of three leaves from each of the normal, N-deficient, and S-deficient plants. This instrument basically measures the emitted energy in the spectral range of 6 to 14  $\mu$ m from which

---

<sup>4</sup> The thermogram and the densitometry measurement were carried out by courtesy of Texas Instruments, Inc., Stafford, Texas.

instant temperature maps or thermograms are produced on a television-type screen and photographed (21). The scanning time used to develop the thermogram was 4.5 seconds. A styrofoam surface was used as a background for the maize leaves. Three densitometer readings were made at the center of the polaroid negative, and the temperature values were determined from a calibrated density scale.

## RESULTS

Results from the analysis of variance for the reflectance, transmittance, and absorbance data at 530 nm and 640 nm show significant differences ( $P = .01$ ) among leaf numbers 3, 4, and 5, among the seven nutrient treatments, and among the interactions of leaves and treatments. The spectral variation in these visible wavelengths was influenced by the physiological age of leaves as well as the plant nutrient deficiencies.

The Scheffe test for multiple comparisons was run to determine significant differences between average spectral response for all pairs of treatments (normal, -N, -P, -K, -Mg, -Ca, -S) and all pairs of leaves for the reflectance, transmittance, and absorbance data at the 530 and 640 nm wavelengths. The results are summarized by tabular and graphic forms for absorbance only (Table 1, Figure 1). Some of the calculated absorbance variation among the leaves at these wavelengths may be attributable to the impossibility with the spectrophotometer of scanning exactly the same leaf area for both transmittance and reflectance measurements.

The highest absorbance values were obtained in all cases for the leaves of normal, Ca- and P-deficient plants. This is in agreement with the chlorophyll content of these leaves as it can be noted from Table 2. The Scheffe multiple comparison test results (Table 1) for both wavelengths also indicates that the means of normal, Ca-, and P- treatments were significantly ( $P = .05$ ) greater than the means of N-, K-, Mg-, and S-deficient leaves. Other comparisons of means were included and in general the results show the effect of nutrient deficiencies on the spectral characteristics of the maize throughout the visible region of the spectrum.

The average chlorophyll content, leaf thickness, and percent leaf moisture of leaves 3, 4, and 5 were determined for each of the seven nutrient treatments (Table 2). All nutrient-deficient plants contained less

chlorophyll than the control. These results indicate that chlorophyll has a dominant influence on spectral variations in the visible region of the spectrum.

Results of the analysis of variance for the spectral reflectance, transmittance, and absorbance of the remaining twelve wavelengths at the near and the reflective infrared regions were found to be very similar. In all cases only the variation due to treatments was found to be significant ( $P = 0.01$ ).

It is also important to emphasize that leaf physiological age does not seem to influence the spectral characteristics of leaves of the same nutrient treatment in these spectral regions.

The Scheffe test for absorbance shows that only Mg-deficiency is significantly ( $P = .05$ ) greater than P-deficiency at both 830 and 940 nm wavelengths, but no significant differences were shown for other deficiencies. No significant differences were also obtained among all seven treatments at 1100 and 1200 nm wavelengths.

The Scheffe test results show that S-deficient leaves had the highest level of absorbance among all deficiencies at wavelengths 1400, 1430, 1450, 1550, 1700, 1930, 2000 and 2180 nm. Nitrogen and Mg-deficiencies showed second highest absorbance to all other deficiencies. These results correspond to the leaf moisture content shown in Table 2.

The average absolute values of the spectral reflectance, transmittance of leaves 3, 4, and 5 and for each of the seven treatments were plotted for the wavelengths between 500 and 2600 nm (Figure 2 and 3).

The area between % transmittance and % reflectance of these two figures is % absorbance. However, higher reflectance associated with lower transmittance or vice versa is expected since the sum of the three variables is unity.

The average percent absorbance for leaves 3, 4, and 5 at 830, 940, and 1100 nm wavelength for all the treatments was calculated (Table 3).

A thermogram representing temperature profiles of maize leaves of the control, S-deficient and N-deficient plants was obtained (Figure 4). Film density measurements were made on the thermogram, and calibrated equivalent temperature values were calculated (Table 4).

Leaf thickness versus leaf moisture content were plotted (Figure 5). Polynomial regression analysis was used to study the relationship between these two variables. A second degree polynomial was fitted to the data with an  $R^2$  value of 0.80.

## DISCUSSION

Solar radiation in the electromagnetic wavelength range from 500 to 2600 nm which reaches the earth may be absorbed, transmitted and reflected by plant leaves (6).

The spectral characteristics of the leaf in the visible region of the spectrum (500 - 750 nm) are associated mainly with leaf pigments (6, 17). Benedict and Swindler (3), working with soybeans and citrus, and Thomas and Oerther (23), working with sweet peppers, found an inverse relationship between reflectance and chlorophyll content.

The metabolic disturbances resulting from nutrient deficiencies of maize in this experiment lead to a reduction of leaf chlorophyll content and consequently the alteration of leaf color, reflectivity, and transmittance. These conditions are clearly illustrated (Figures 2 and 3). N-deficient maize has the least amount of chlorophyll and is followed in order of increasing chlorophyll content by -S, -Mg, -K, -Ca, -P, and normal maize plants (Table 1).

Reflectance and transmittance of the leaf in the near-infrared region (750 - 1300 nm) is generally associated with leaf structure and morphology. In young or immature leaves low reflectance and high transmittance is expected because cells are small and there are few intercellular spaces. As the leaf matures, differentiation into palisade and spongy mesophyll layer becomes more pronounced, intercellular spaces and vacuoles increase in size, and consequently reflectance increases. However, in monocots like maize there is no distinct organization of the mesophyll into palisade and spongy mesophyll layers (9). Myers et al. (16) have associated the severity of nitrogen-deficient sweet pepper leaves with increased near-infrared reflectance. They attribute this increase in reflectance to smaller and fewer cells within the leaf. In the N-deficient maize leaves of this study,

cells were smaller and chloroplasts fewer in number than in the control (A. H. Al-Abbas, unpublished data). Similar results were obtained with S- and Mg-deficient maize leaves.

Among all treatments the K-deficient leaves gave the highest reflectance and had the lowest leaf thickness and least leaf moisture content. In addition to cell size and altered structure, reflectance seems to be related closely with leaf thickness and moisture content.

It is interesting to note (Table 3) that the percent absorbance of -P and -Ca leaves at the wavelengths 830, 940, and 1100 nm was considerably lower than the absorbance value for the control plant. Conversely, the absorbance of the -S, -Mg, -K, and -N leaves were much higher than that of the control. The low energy absorbance is associated with those treatments which seemingly have a high chloroplast number of a high chlorophyll content. The higher incident energy absorption at these wavelengths by -S, -Mg, -K, and -N treatments may result from a higher heat content within their leaves, and they may have a substantially higher temperature than do control, P-deficient and Ca-deficient plants. The low absorbance function of this region (near-infrared) will reduce the incident solar energy absorbed by the leaf and consequently protect the plant pigments from denaturation. However, the growth abnormality resulting from these nutrient deficiencies may affect stomatal development and transpiration which may lead to increased leaf temperature (5, 19).

The amount of energy a leaf may radiate is dependent upon its temperature and the emitted radiation is spectrally distributed as a function of that temperature (5). The thermogram (Figure 3) illustrates the variation in temperature between control, sulfur-deficient and nitrogen-deficient maize leaves. The average densitometry readings translated to temperature values (Table 4) confirm the visual observation from the thermogram which indicates that nitrogen-deficient leaves are warmer by  $0.9^{\circ}\text{C}$  and sulfur by  $0.4^{\circ}\text{C}$  than normal leaves. Gates (5) found an increase of  $0.5^{\circ}$  to  $1.5^{\circ}\text{C}$  in K-deficient sugarcane leaves compared to normal ones when both were exposed simultaneously to sunlight.



The spectral reflectance and transmittance in the wavelength interval between 1300 and 2600 nm is related mainly to leaf water content (17). Although the fundamental water absorption region is between 2600 and 2800 nm, liquid water overtones affected reflectance strongly at 1450 and 1930 bands which were significantly ( $P = .01$ ) related to relative turgidity or water content of cotton leaves. Gausman et al. (19) obtained a positive correlation between cotton leaf water content and the absorbance coefficient ( $K$ ) at 1950 nm. An examination of the water content of the various nutrient-deficient maize leaves in this study showed a positive correlation ( $r = 0.707$ ) between the water content and percent absorbance at 1450 and 1930 nm wavelength where both reflectance and transmittance are inversely proportional to leaf water content.

In summary, the reduction of leaf chlorophyll content has introduced a wide spectral variation among the nutrient-deficient plants in the visible region of the spectrum. Nutrient-deficient maize leaves have shown measurable variation in regard to leaf thickness, leaf moisture content, and leaf temperature. These variations have affected the near and far infrared, and the thermal region of the spectrum. Therefore, spectral detection of nutrient-deficient plants is possible by remote sensing techniques.

#### LITERATURE CITED

1. Arnon, D. I., 1949. Copper enzymes in isolated chloroplasts. Polyphenol-oxidase in Beta vulgaris. Plant Physiol. 24:1-15.
2. Barr, R., A. H. Al-Abbas, J. D. Hall and F. L. Crane. 1971. Lipophilic quinones in mineral-deficient maize leaves. Indiana Acad. of Sci. 80:130-139.
3. Benedict, H. M. and R. Swindler. 1961. Nondestructive method for estimating chlorophyll content of leaves. Science 113:2015-2016.
4. Escobar, D. E., R. R. Rodriguez, and H. W. Gausman. 1971. Light reflectance, transmittance and absorbance of nutrient sufficient and deficient squash leaves. 1971 Annual Report, Weslaco, Texas.
5. Gates, D. M. 1970. Physical and physiological properties of plants. In Remote Sensing. National Academy of Sciences, Washington, D. C. pp. 224-252.
6. Gates, D. M., H. J. Kiegan, J. C. Schleiter and V. R. Weidner. 1965. Spectral properties of plants. Applied Optics 4:11-20.
7. Gates, D. M. and W. Tantrapron. 1952. The reflectivity of deciduous trees and herbaceous plants in the infrared to 25 microns. Science 115:613-616.
8. Gausman, H. W., W. A. Allen, V. I. Myers and R. Cardenas. 1969. Reflectance and internal structure of cotton leaves. Agron. J. 61:374-376.
9. Gausman, H. W., W. A. Allen, R. Cardenas and A. J. Richardson. 1970. Relation of light reflectance to histological and physical evaluations of cotton leaf maturity. Applied Optics 9:545-552.
10. Gausman, H. W., W. A. Allen, C. L. Wiegand, D. E. Escobar, R. R. Rodriguez and A. J. Richardson. 1971. The leaf mesophylls of twenty crops, their spectra and optical and geometrical parameters. Annual Report, Weslaco, Texas.
11. Gausman, H. W. and R. Cardenas. 1968. Effect of soil salinity on external morphology of cotton leaves. Agron. J. 60:566-567.
12. Hoagland, D. R. and D. I. Arnon. 1950. The waterculture method for growing plants without soil. Bull. No. 347, California Agr. Exp. Stat., Berkeley, California.
13. Johannsen, C. J. 1969. The detection of available soil moisture by remote sensing techniques. Ph.D. thesis, Purdue University (Diss. Abst. Int. Vol. 30, No. 11, 1970).

14. Knipling, E. B. 1970. Physical and physiological basis for the reflectance of visible and near-infrared radiation from vegetation. *Remote Sensing Environment* 1:155-159.
15. MacLachlan, S. and S. Zalik. 1963. Plastid structure, chlorophyll concentration, and free amino acid composition of a chlorophyll mutant of barley. *Can. J. Bot.* 41:1053-1062.
16. Myers, V. I. 1970. Soil, water and plant relations. In *Remote Sensing*. National Academy of Sciences, Washington, D. C. pp. 253-297.
17. Myers, V. I. and W. A. Allen. 1968. Electrooptical remote sensing methods as nondestructive testing and measuring techniques in agriculture. *Applied Optics* 7:1819-1838.
18. Ostle, B. 1962. *Statistics in research*. Iowa State College Press, 2nd Edition.
19. Shugarov, Y. A. 1967. The influence of potassium on the absorption of near infrared radiation by plant leaves. *Potash Review*. (April), (Agrokhimiya No. 11:76, 1966, Moscow).
20. Sinclair, T. R., R. M. Hoffer and M. M. Schreiber. 1971. Reflectance and internal structure of leaves from several crops during a growing season. *Agron. J.* 63:864-868.
21. Texas Instruments. 1970. *An introduction to medical thermography*. Texas Instrument reprint.
22. Thomas, J. R., I. N. Nenken, G. F. Oerther and R. G. Brown. 1971. Estimating leaf water content by reflectance measurements. *Agron. J.* 63:845-847.
23. Thomas, J. R. and G. F. Oerther. 1972. Estimating nitrogen content of sweet pepper leaves by reflectance measurements. *Agron. J.* 64:11-13.

#### TABLE LEGENDS

- Table 1. Scheffe' multiple comparison test results for absorbance of maize leaves 3, 4, and 5, respectively.
- Table 2. Average values for chlorophyll content, moisture content, and leaf thickness for normal and the six nutrient-deficient treatments of maize leaves.
- Table 3. Average percent absorbance for normal and six nutrient-deficient treatments of maize leaves.
- Table 4. Maize leaf temperature in degrees centigrade as calibrated from thermogram density.

Table 1. Scheffe' multiple comparison test results for absorbance of maize leaves 3, 4, and 5, respectively.

Wavelength	Leaf #3	Leaf #4	Leaf #5
530 nm	Norm., -a>* -N, -K, -S  -N, -P, -Mg>* -S	Norm., -P, -Ca>* -N -K, -Mg, -S	Norm., -Ca>* -N, -Mg -S  -P >* -N, -S -K > -N
640 nm	Norm. >* -N, -P, -K -Mg, -S  -Ca >* -P, -K, -Mg, -S -P >* -Mg	Norm. >* -N, -K, -Mg -S  -Ca >* -K, -Mg, -S -P >* -K, -Mg	Norm. >* -N, -K, -Mg -S  -P, -Ca >* -N

>\* = significantly greater than (P = .05).

Table 2. Average values for chlorophyll content, moisture content, and leaf thickness for normal and the six nutrient-deficient treatments of maize leaves.\*

Nutrient Treatments	Chlorophyll Content mg/gm wet weight	Leaf Thickness mg/cm <sup>2</sup>	Percent Moisture
Control	1.156	9.2	68.5
-P	1.023	4.0	49.7
-Ca	0.866	6.7	58.2
-K	0.625	4.7	46.4
-S	0.455	11.0	82.4
-Mg	0.433	9.0	73.2
-N	0.205	9.4	72.7

\* Average values for leaves 3, 4, and 5.

Table 3. Average percent absorbance for normal and six nutrient-deficient treatments of maize leaves.

Treatment	% Absorbance*		
	830 nm	940 nm	1100nm
Control	2.37	2.92	3.66
-P	0.92	1.02	1.21
-Ca	1.71	1.40	2.10
-K	5.28	5.23	4.74
-S	5.57	5.74	7.11
-Mg	7.29	7.19	6.83
-N	4.72	4.91	5.50

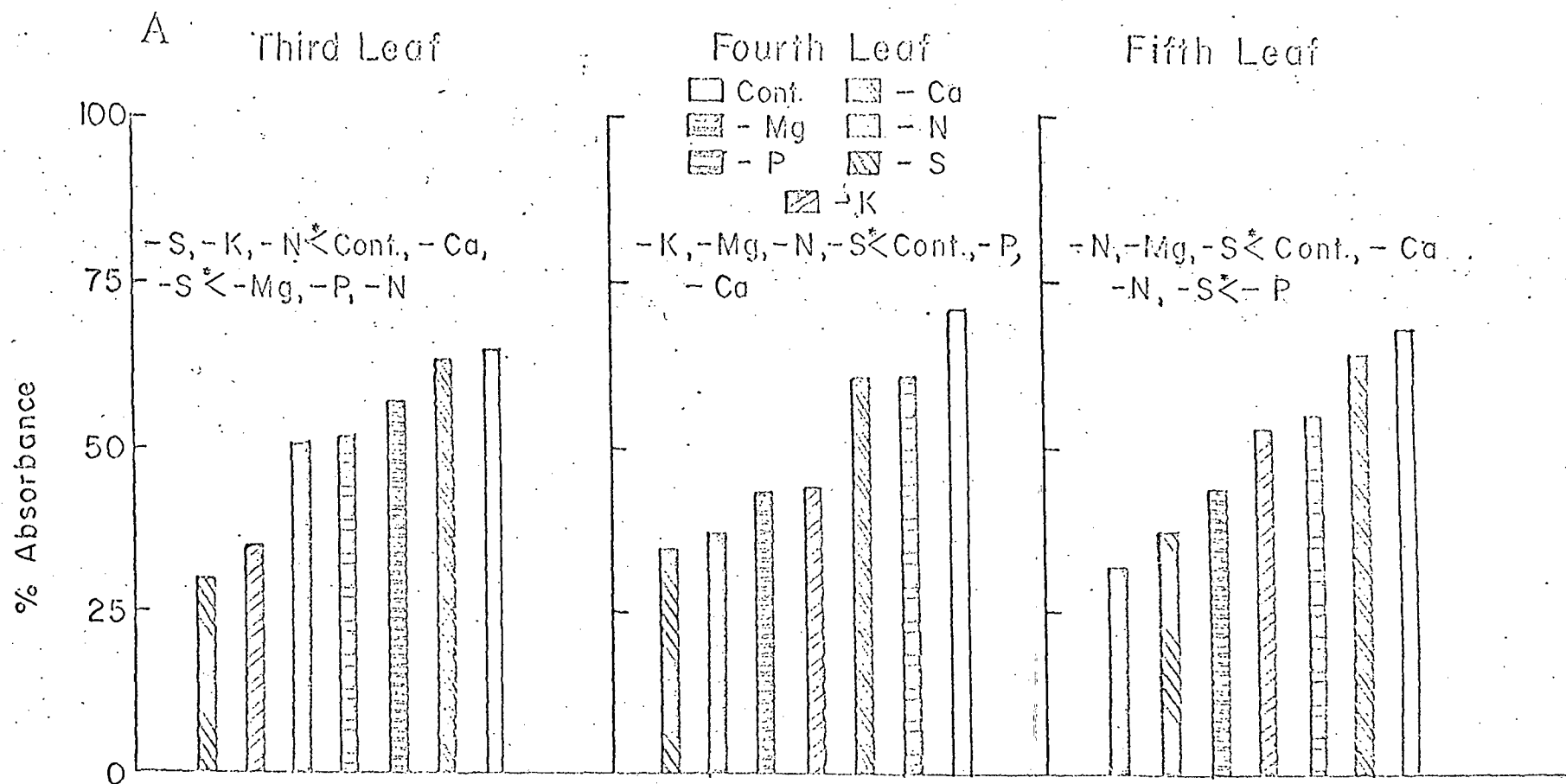
\* Average values for leaves 3, 4, and 5.

Table 4. Maize leaf temperature in degrees centigrade  
as calibrated from thermogram density.

Trial	Leaf Treatments - Temp. °C								
	Control			-S			-N		
	1	2	3	1	2	3	1	2	3
1	27.44	26.83	27.01	26.79	27.40	27.98	28.45	28.01	28.08
2	27.16	27.01	27.15	27.55	27.29	28.23	28.45	27.69	28.08
3	20.07	27.04	27.58	27.15	27.15	27.73	27.83	27.91	27.98
Average	27.22	26.94	27.22	27.16	27.27	28.00	28.22	27.88	28.05
Mean	27.11			27.50			28.05		



Fig. 4. 1. 11



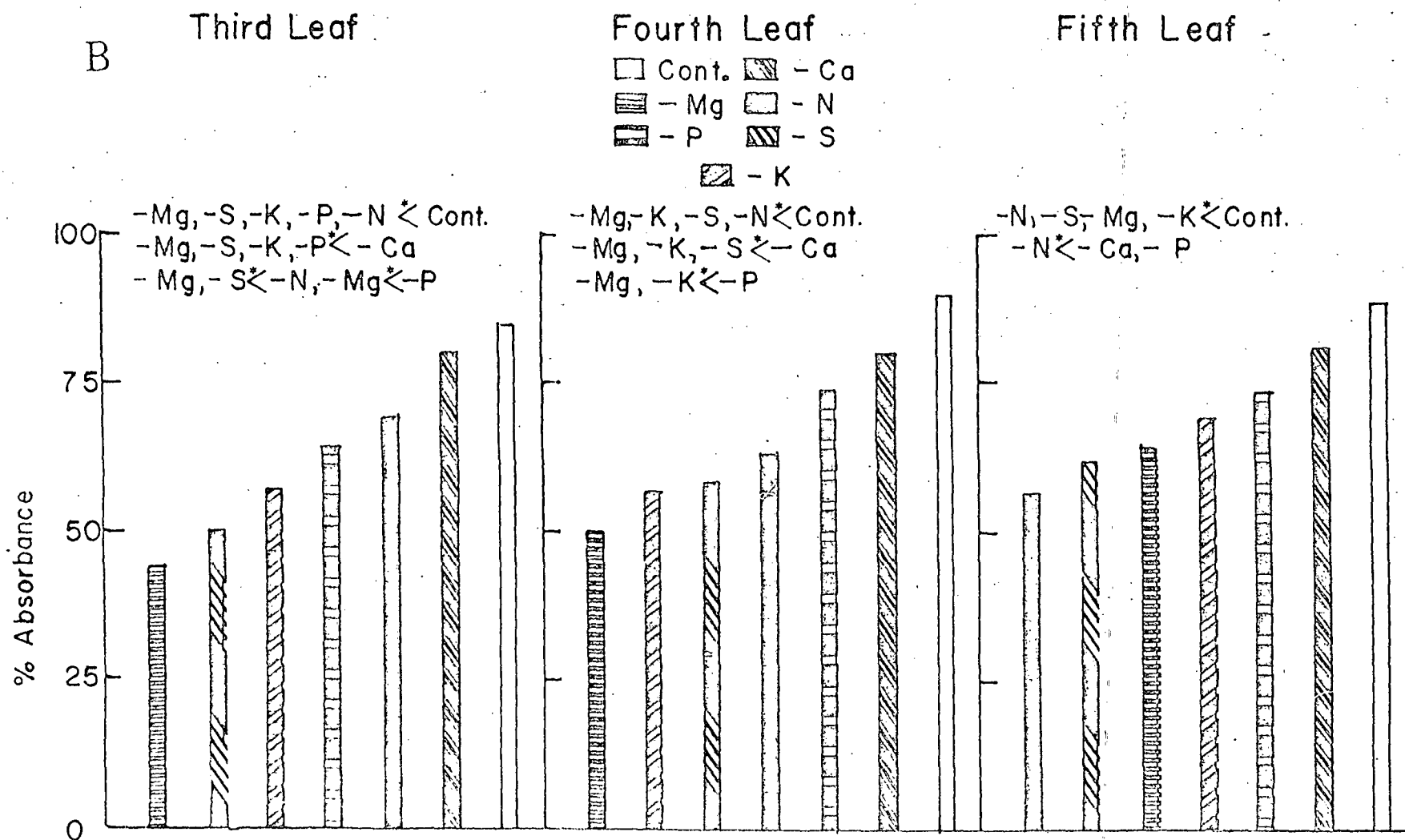


Figure 1-B

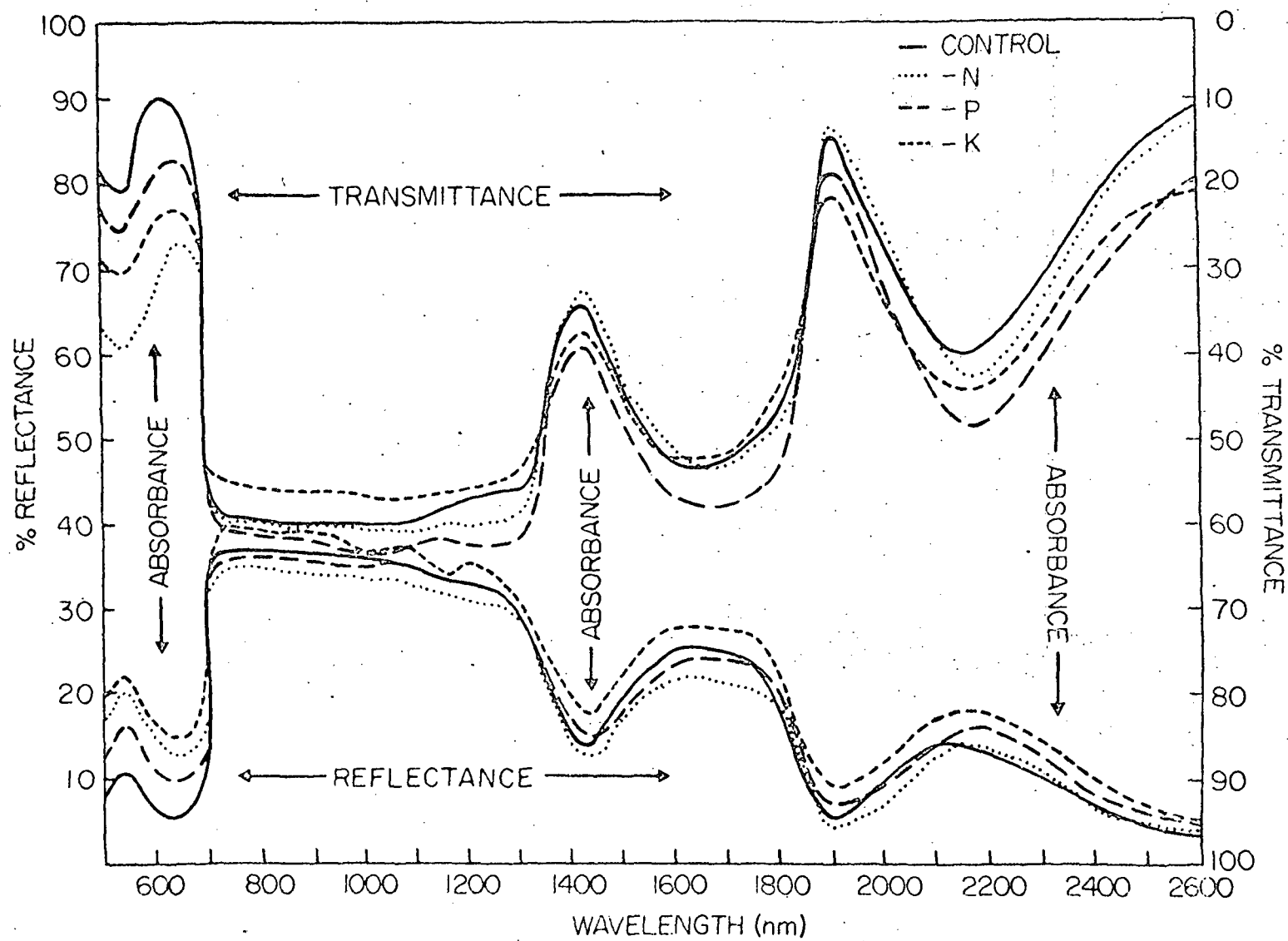
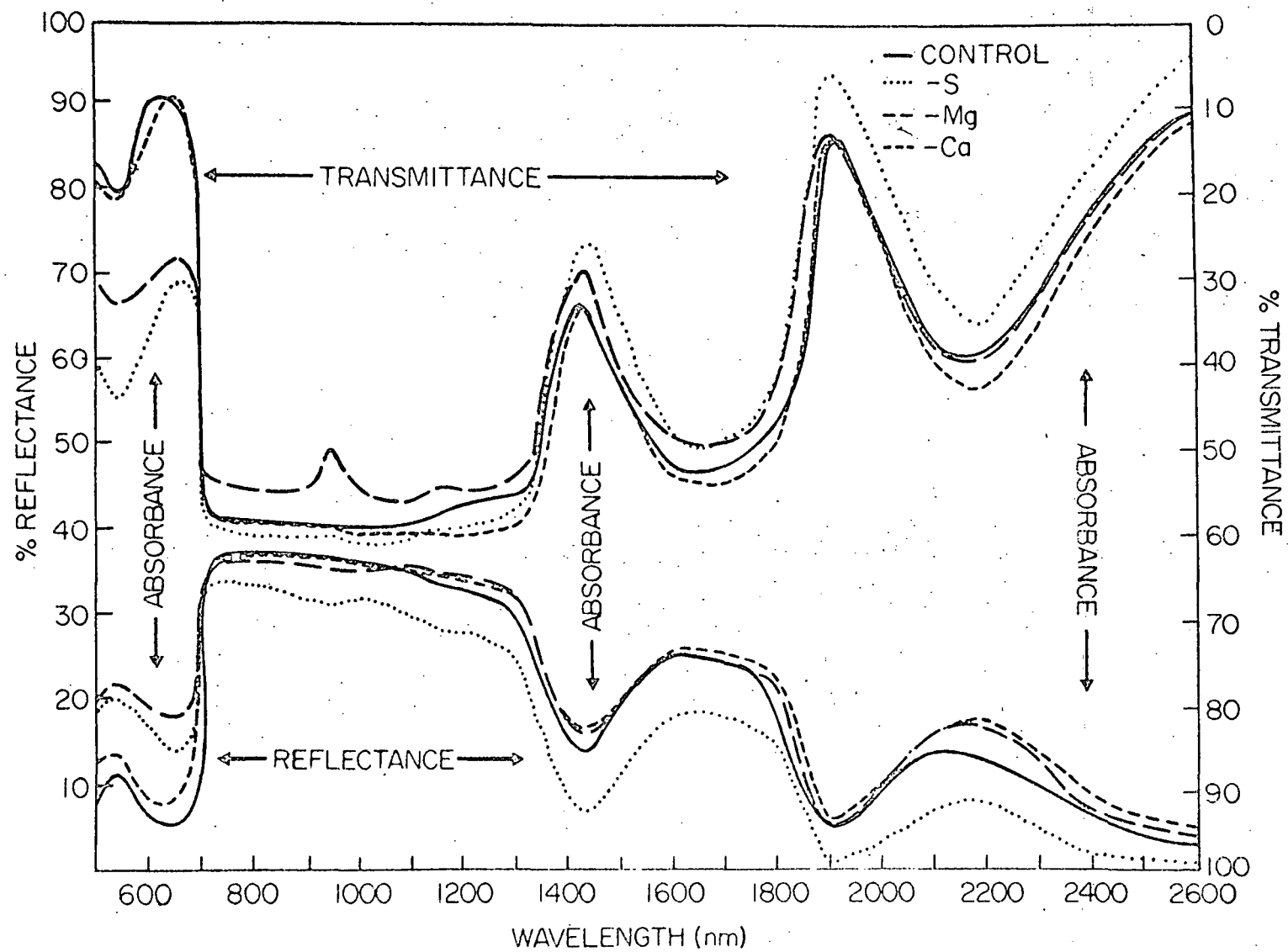


Figure 2

Figure 3



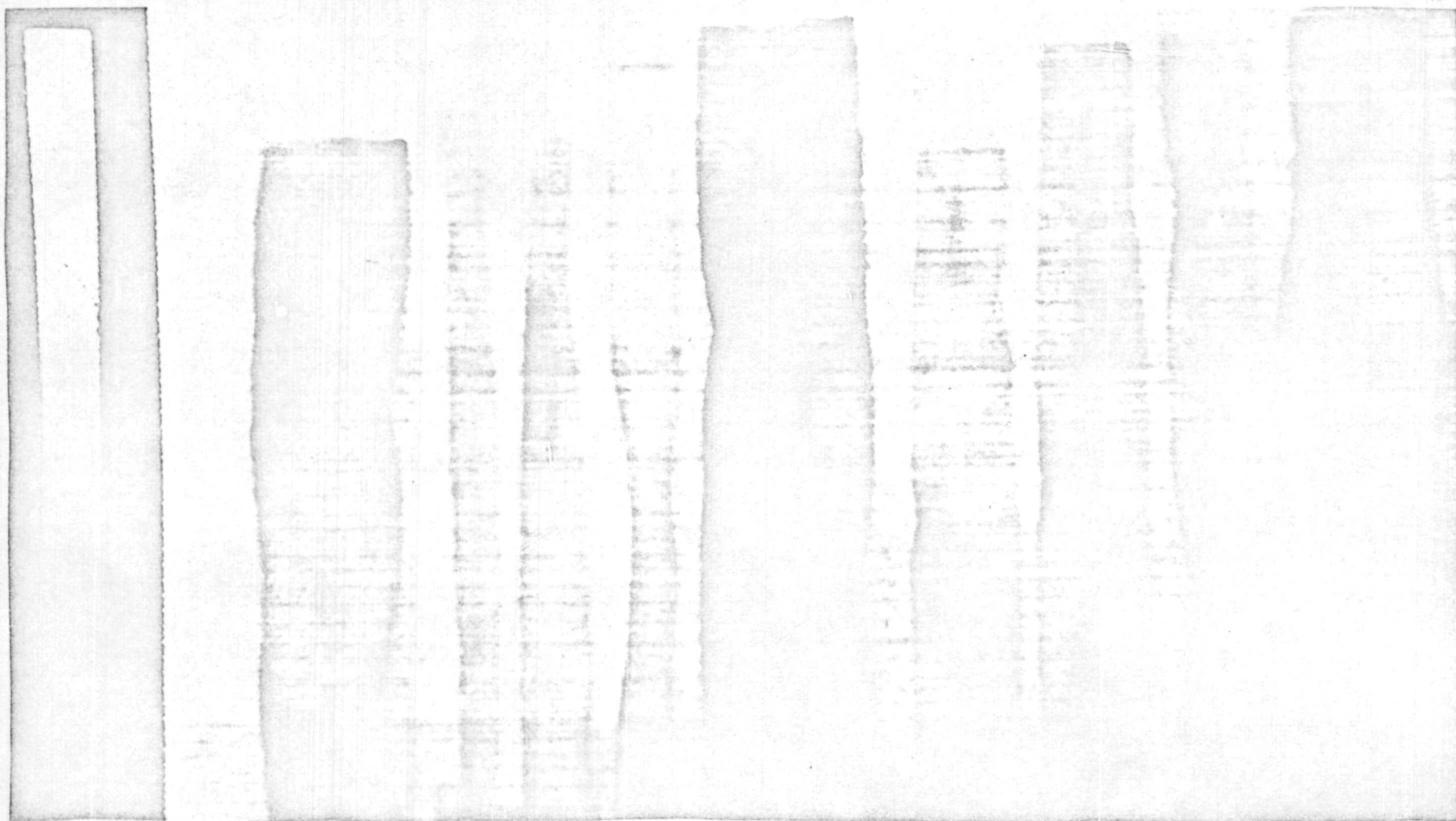


Figure 4

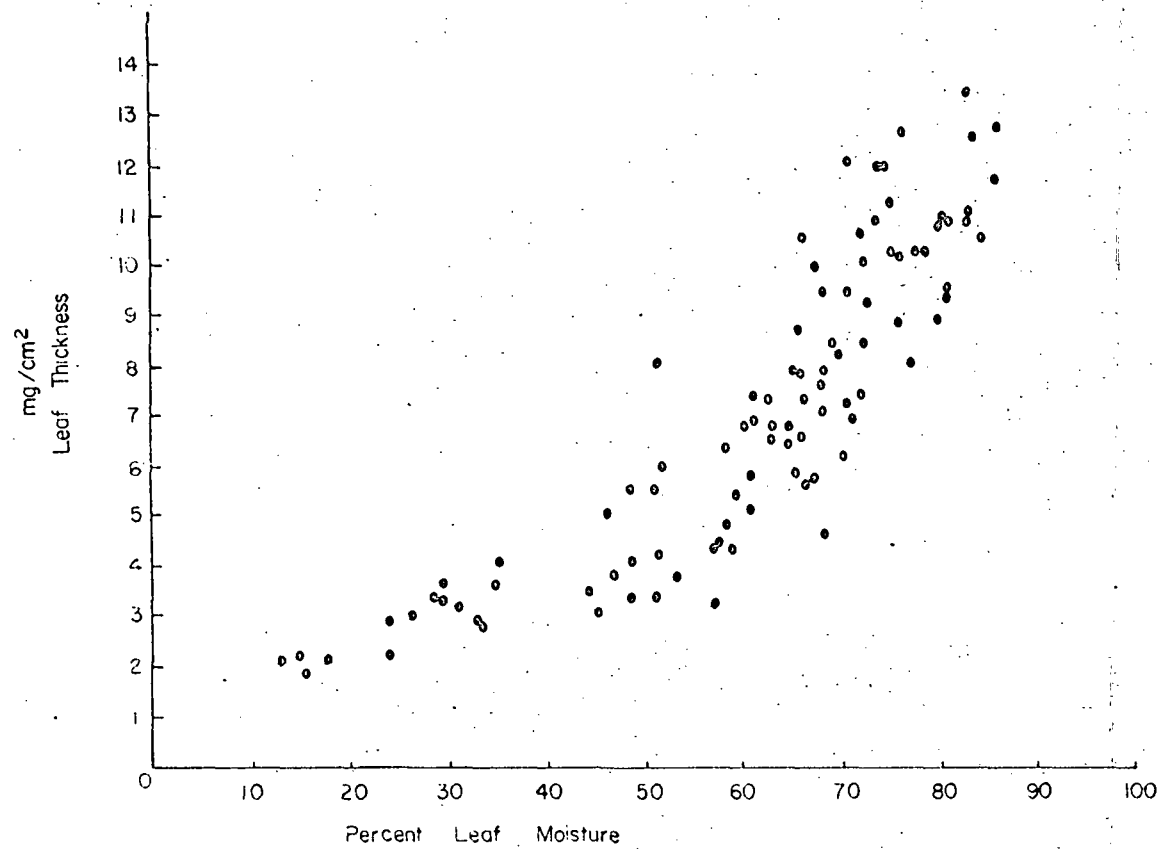


Figure 5